

WHAT IS CLAIMED IS:

1. A method of detecting chemiluminescent emissions on a solid support,
the method comprising:

5 contacting a surface layer of the solid support with a substrate composition
comprising a first chemiluminescent substrate capable of being activated by a first
enzyme to produce a first chemiluminescent signal and a second chemiluminescent
substrate capable of being activated by a second enzyme to produce a second
chemiluminescent signal; and

10 detecting first and second chemiluminescent signals on the surface layer of
the solid support;

 wherein a plurality of probes are disposed in a plurality of discrete areas on
the surface layer at a density of at least 50 discrete areas per cm², wherein at least
some of the probes are bound to a first enzyme conjugate comprising the first
enzyme, and wherein at least some of the probes are bound to a second enzyme
15 conjugate comprising the second enzyme.

2. The method of Claim 1, wherein the composition comprising the first
and second chemiluminescent substrates is contacted with the surface layer in the
presence of a composition comprising a chemiluminescent quantum yield
enhancing material.

20 3. The method of Claim 1, wherein the discrete areas comprise one or more
control probes and wherein the first enzyme conjugate is bound to a control probe.

 4. The method of Claim 3, further comprising quantifying the amount of
the second chemiluminescent signal.

5. The method of Claim 4, wherein quantifying comprises comparing the intensity of the first chemiluminescent signal to the intensity of the second chemiluminescent signal.

6. The method of Claim 3, wherein a plurality of different probes are disposed on the support surface in different discrete areas and wherein detecting comprises detecting the location on the support surface of first and second chemiluminescent signals.

7. The method of Claim 6, wherein control probes are located in one or more discrete areas on the support surface.

8. The method of Claim 6, wherein control probes are co-located in one or more of the same discrete areas as probes for a target molecule.

9. The method of Claim 1, wherein detecting comprises detecting the location on the support surface of first and second chemiluminescent signals.

10. The method of Claim 9, wherein the plurality of discrete areas comprise oligonucleotide or nucleic acid probes.

11. The method of Claim 1, further comprising:
contacting the support surface with a sample comprising first target molecules labeled with a first label and second target molecules labeled with a second label prior to contacting the support surface with the substrate composition.

12. The method of Claim 11, wherein the first target molecules are labeled with the first enzyme to form the first enzyme conjugate and the second target molecules are labeled with the second enzyme to form the second enzyme conjugate.

13. The method of Claim 11, wherein the first target molecules are labeled with a moiety capable of binding to the first enzyme conjugate and the second target molecules are labeled with a moiety capable of binding to the second enzyme conjugate.
- 5 14. The method of Claim 11, wherein the first target molecules comprise a first pool of target nucleic acids and wherein the second target molecules comprise a second pool of target nucleic acids.
15. The method of Claim 14, wherein the first and second pools of target nucleic acids each comprise mRNA transcripts of one or more genes or nucleic acids derived from mRNA transcripts of one or more genes.
- 10 16. The method of Claim 14, wherein the first and second pools of target nucleic acids each comprise cDNA or cRNA derived from mRNA transcripts.
17. The method of Claim 14, wherein the concentration of the target nucleic acids in the first and second pools of target nucleic acids is proportional to the expression level of the genes encoding the target nucleic acid.
- 15 18. The method of Claim 11, wherein the probes comprise a control probe and wherein the first enzyme conjugate is bound to the control probe.
19. The method of Claim 18, wherein the plurality of different probes comprise oligonucleotide or nucleic acid probes and wherein the sample comprises a pool of target nucleic acids labeled with the second enzyme.
- 20 20. The method of Claim 19, wherein the pool of target nucleic acids comprises mRNA transcripts of one or more genes or nucleic acids derived from the mRNA transcripts of the one or more genes.

21. The method of Claim 20, wherein the pool of target nucleic acids comprises cDNA or cRNA derived from mRNA transcripts of the one or more genes.
22. The method of Claim 21, wherein the concentration of each of the target nucleic acids in the pool of target nucleic acids is proportional to the expression level of each of the genes encoding the target nucleic acid.
23. The method of Claim 1, wherein the density of discrete areas on the surface layer is at least 100 discrete areas per cm^2 .
24. The method of Claim 1, wherein the density of discrete areas on the surface layer is at least 1,000 discrete areas per cm^2 .
25. The method of Claim 1, wherein the density of discrete areas on the surface layer is at least 25,000 discrete areas per cm^2 .
26. The method of Claim 1, wherein the density of discrete areas on the surface layer is at least 50,000 discrete areas per cm^2 .
27. The method of Claim 1, wherein the support surface further comprises a fluorescent control.
28. The method of Claim 1, wherein the first chemiluminescent signal and the second chemiluminescent signal have different emission maxima.
29. The method of Claim 28, wherein detecting first and second chemiluminescent signals comprises:
- filtering the emissions from the support surface with a first filter adapted to reduce the intensity of the second chemiluminescent signal relative to the intensity of the first chemiluminescent signal;

detecting the first chemiluminescent signal;

filtering the combined signal from the support surface with a second filter adapted to reduce the intensity of the first chemiluminescent signal relative to the intensity of the second chemiluminescent signal; and

5 detecting the second chemiluminescent signal.

30. The method of Claim 1, wherein the composition comprising the first and second chemiluminescent substrates is a buffered solution.

31. The method of Claim 1, further comprising washing the surface layer of the solid support before contacting the surface layer with the substrate
10 composition.

32. The composition of Claim 1, wherein the first and second chemiluminescent substrates are both 1,2-dioxetanes.

33. The composition of Claim 1, wherein the first chemiluminescent substrate is a 1,2-dioxetane substrate and the second chemiluminescent substrate is
15 selected from the group consisting of an acridan ester substrate, an acridan thioester substrate, an enol phosphate substrate, an acridan enol phosphate substrate, and a luminol substrate.

34. A composition comprising a first chemiluminescent substrate capable of being activated by a first enzyme to produce a first chemiluminescent signal and
20 a second chemiluminescent substrate capable of being activated by a second enzyme to produce a second chemiluminescent signal, wherein the first and second chemiluminescent signals are different.

35. The composition of Claim 34, wherein the composition is a buffered solution.

36. The composition of Claim 34, further comprising a chemiluminescent quantum yield enhancing agent, additive and/or counterion.

5 37. The composition of Claim 34, wherein the first and second chemiluminescent substrates are each 1,2-dioxetanes.

38. The composition of Claim 34, wherein the first chemiluminescent substrate is a 1,2-dioxetane substrate and the second chemiluminescent substrate is selected from the group consisting of an acridan ester substrate, an acridan
10 thioester substrate, an enol phosphate substrate, an acridan enol phosphate substrate, and a luminol substrate.

39. The method of Claim 2, wherein the composition comprising the chemiluminescent quantum yield enhancing material further comprises an additive selected from the group consisting of BSA, cyclodextrins, negatively charged salts,
15 alcohols, polyols, poly(2-ethyl-Z-oxazoline), zwitterionic surfactants, anionic surfactants, cationic surfactants, and neutral surfactants.

40. The method of Claim 2, wherein the composition comprising the chemiluminescent quantum yield enhancing material further comprises one or more counterion moieties selected from the group consisting of halide, sulfate,
20 alkylsulfonate, triflate, arylsulfonate, perchlorate, alkanoate, arylcarboxylate and combinations thereof.

41. The method of Claim 13, wherein the first or second enzyme conjugate is an antidigoxigenin:enzyme conjugate and wherein the corresponding target

molecules are labeled with digoxigenin.

42. The method of Claim 14, wherein the first or second pools of target nucleic acids are labeled with digoxigenin and the corresponding enzyme conjugate is an antidigoxigenin:enzyme conjugate.

5 43. The method of Claim 42, wherein the pool of target nucleic acids labeled with digoxigenin comprises cDNA.

44. The method of Claim 2, wherein the chemiluminescent quantum yield enhancing material is an onium polymer selected from the group consisting of poly(vinylbenzylammonium salts), poly(vinylbenzylphosphonium salts) and
10 poly(vinylbenzylsulfonium salts).

45. The method of Claim 2, wherein the chemiluminescent quantum yield enhancing material is an onium copolymer.